Status and Final Technical Report

Grant: **NAGW-3807 (4 month extension of NAG-10-0061)**

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Project Title: **Calcium/Calmodulin-mediated Gravitropic Response in Plants**

Calmodulin isoforms: We have cloned and characterized eight calmodulin isoforms and identified one isoform (*PCM-1*) that is highly responsive to changes in the environment. A transgene approach was taken to study the consequences of altered expression of *PCM-1* on plant growth and development. Transgenic potato plants were produced carrying sense and antisense constructs of *PCM-1* fused to the CaMV 35S promoter. Transgenic sense plants showing a moderate increase in *PCM-1* mRNA exhibited strong apical dominance, produced elongated tubers, and were taller than the controls (Patent No. 5,498,533, 1996). Interestingly, the plants expressing the highest level of *PCM-1* mRNA did not form underground tubers; instead, these transgenic plants produced aerial tubers when allowed to grow for longer periods. The formation of aerial tubers in these transgenic sense plants is an indication that the inhibitory effect of light on tuberization has been altered. Transgenic plants were also produced carrying sense and antisense constructs of *PCM-1* fused to an inducible patatin promoter. The antisense plants grew similar to the control plants in the initial stages of growth. However, in the later stages of growth when the patatin promoter became active, growth was reduced and the plants exhibited leaf burns. The control plants grown under identical conditions did not show leaf burns, suggesting an altered response of transgenic plants to the environmental conditions. To further study the role of *PCM-1* in signal transduction, transgenic potato plants carrying the *PCM-1* promoter fused to the GUS reporter gene were produced. GUS expression was found to be developmentally regulated and signal-responsive, indicating a positive correlation between the expression of *PCM-1* and GUS mRNAs. These results suggest that the 5' flanking region of *PCM-1* controls developmental and signal-induced expression. We have also expressed *PCM-1* and *PCM-6* isoforms in *E. coli* and purified the proteins. These proteins showed different mobility patterns on SDS-PAGE which can be recognized by western analysis.

Novel calmodulin-binding proteins: To clone and characterize cDNAs that encode for calmodulin-binding proteins, ^{35}S-labeled mammalian calmodulin was used. Since the clones that were obtained did not show any sequence similarity to known genes, we used purified recombinant ^{35}S-labeled potato calmodulin as a ligand probe to screen cDNA expression libraries from different tissues. Several positive clones were obtained and two had homology to known genes. The sequence comparison and characterization revealed that one of the clones *(TCK1)* has homology to kinesin-like proteins. TCK1 is unique in its ability to bind to calmodulin in the presence of Ca^{2+}. The presence of a CaM-binding domain within the motor domain of TCK1 makes it a unique addition to the kinesin superfamily.

We have cloned and characterized a chimeric Ca^{2+}/calmodulin-dependent protein kinase gene with a neural visinin-like Ca^{2+}-binding domain. The biochemical characterization of this novel kinase (CCaMK) revealed that it is modulated by Ca^{2+} and Ca^{2+}/calmodulin. CCaMK contains

all eleven major conserved subdomains of the catalytic domain of serine/threonine kinases. Sequence comparisons revealed that CCaMK has high similarity to mammalian Ca^{2+}/CaM-dependent protein kinases, especially in the kinase and CaM-binding domains (amino acid residues 1-338). The CaM-binding region of CCaMK (FNARRKLRAAAIASVL, residues 323-338) is similar to the CaM-binding domain (FNARRKLKGAILTTML, residues 293-309) of the α-subunit of mammalian CaMKII. The sequence downstream of the CaM-binding region of CCaMK (amino acid residues 339-520) does not have significant similarity to known Ca^{2+}/CaM-dependent protein kinases. Further analysis of this region revealed the presence of three Ca^{2+}-binding EF-hand motifs that had high homology (52-54% similarity; 32-35% identity) to frequenin, neurocalcin, hippocalcin, and visinin-like neural Ca^{2+}-binding proteins. These proteins are members of a family of Ca^{2+} sensitive regulators, each containing three Ca^{2+}-binding EF-hand motifs. The structural features of CCaMK indicate that it is a chimeric Ca^{2+}- and Ca^{2+}/CaM-dependent protein kinase with two distinct regulatory domains; a CaM-binding domain and a visinin-like Ca^{2+}-binding domain. We have compared the CCaMK sequences of monocotyledonous (lily) and dicotyledonous (tobacco) plants and they share 66% identity and 79% similarity in the kinase domain (amino acid residues 1 to 307). However, the 3' visinin-like domain (amino acid residues 339 to 517) shares 79% identity and 87% similarity; suggesting that the visinin-like domain is conserved and may play an important role in regulating CCaMK activity.

Publications Resulting from NASA Award

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